Platelet Aggregation and ATP Release from Dense Platelet Granules in Immobilized Rats

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UDC 616.155.25-008.939.633.2-0.92.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 7, pp. 40-42, July, 1993
Original article submitted February 9, 1993.

Key Words: *immobilization; open-field behavior; platelets aggregation; ATP; ADP; collagen, epinephrine*

There is clinical and experimental evidence indicating that disorders of circulation, in whose origin an important role is played by altered platelets, rank prominently among diseases associated with emotional stress. In addition to being directly implicated in thrombus formation and the development of atherosclerotic lesions [10], platelets release substances that locally influence vascular tone [14,17], augment vascular responses to vasoactive agents [4], alter the sensitivity of vascular receptors, and stimulate synthesis of vasoactive peptides [15] in blood vessel walls.

Although unequivocal morphological evidence that intravascular platelet aggregation is increased in rats during stress was provided about two decades ago [8,9], information on changes in platelet function that occur in stressed rats is scarce and contradictory.

In this work, we examined the aggregation of platelets and the release of ATP from their dense granules (as an integral index of degranulation) in rats under standard conditions of immobilization stress (IS). Since IS increases sympathetic tonus and catecholamine release, platelet responses were studied in the presence of epinephrine. The experiments were carried out on August rats, a strain highly susceptible to emotional stress [1]. In addition, we considered the effects of IS in relation to the results of preliminary (prior to the IS) observations of the behavior displayed by the same rats in an open field, since individual behavioral characteristics of rats are known to affect their susceptibility to emotional stress [2,7,16].

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MATERIALS AND METHODS

Forty adult male August rats weighing 224.4±5.0 g were first tested for behavior in an open field, after which they were subjected to stress by being immobilized in close-fitting plastic boxes for 6 or 24 h. Control rats of the same strain were either kept in their cages (1 rat per cage) during the same periods (controls 1) or deprived of food and water for 24 h (controls 2).

Blood samples were taken, under ether anesthesia, from the abdominal aorta into a plastic syringe 10-15 min after the IS and weighing of the animals. Platelets were collected after centrifugation of heparinized blood (200 g for 12 min at 22°C) and diluted with autologous plasma to a concentration of 5×10^8 platelets per ml.

Platelet aggregation and ATP release were assayed in thermostatically controlled (37±0.2°C) cuvettes of an aggregometer (PICA Lumi-Aggregometer, Chrono-log, Havertown, PA, USA). Aggregation was induced with ADP (Sigma) in a concentration of 0.25, 0.5, 1.0, or 5.0 μmol/liter and also with collagen (Chrono-log) in concentrations of 2.0 or 4.0 μg/ml. Platelet aggregation was

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TABLE 1. Platelet Aggregation Rates (%/min). The Values are Means±SEM

Preliminary addition of epinephrine (mmol/liter)	Inducer	Controls 1	Controls 2 (food- and water-deprived rats)	Rats stressed by immobilization	
				for 6 h	for 24 h
		ADP (μmol/liter)			
None	0.25	108.2±11.9 (12)	90.7±17.0 (7)	87.8±26.4 (5)	64.4±14.9 (10)**
1.0	0.25	109.1±8.5 (7)	122.8±24.4 (4)	–	97.5±13.1 (8)+
0.1	0.25	120.0±10.4 (5)	71.5±20.5 (2)	80.3±40.2 (3)	79.5±5.5 (2)
None	0.5	158.1±7.3 (14)	140.0±17.5 (6)	141.0±10.6 (8)	126.0±9.6 (10)**
1.0	0.5	148.4±14.5 (7)	155.8±25.7 (4)	–	132.4±7.1 (8)
0.1	0.5	172.3±16.2 (4)	114.0±18.0 (2)	151.0±19.7 (4)	114.5±5.5 (2)
None	1.0	161.1±11.9 (14)	169.2±18.1 (5)	156.8±12.6 (6)	140.2±11.3 (9)
1.0	1.0	197.3±15.5 (4)	–	–	165.0±4.9 (3)
0.1	1.0	176.5±10.9 (4)	–	157.8±14.9 (4)	–
None	5.0	177.3±9.6 (15)	168.1±16.8 (7)	169.7±10.1 (7)	153.5±7.3 (11)*
1.0	5.0	180.0±9.0 (3)	–	–	154.7±5.8 (3)
0.1	5.0	186.8±7.0 (4)	–	184.0±11.1 (3)	–
		Collagen(μg/ml)			
None	2.0	59.8±8.6 (16)	49.3±12.4 (7)	50.8±18.8 (8)	33.1±6.0 (11)*
1.0	2.0	80.0±11.7 (7)	83.5±13.2 (4)	–	73.0±12.1 (6)+
0.1	2.0	69.7±18.3 (6)	42.0±22 (2)	62.8±24.2 (5)	37.3±9.9 (3)
None	4.0	100.0±9.4 (13)	86.2±12.5 (6)	72.3±20.2 (6)	74.0±8.0 (12)**
1.0	4.0	102.0±9.9 (6)	106.0±13.0 (3)	–	88.0±12.8 (6)+
0.1	4.0	97.0±3.0 (2)	–	59.0±11.0 (2)	64.5±7.5 (2)

Note. Here and in Table 2: *$p<0.05$, **$p<0.025$ in comparison with controls by Mann–Whitney's test; +$p<0.05$ in comparison with rats not injected with epinephrine. Figures in parentheses indicate the number of rats.

also examined in the presence of 0.1-1.0 mmol/liter l-epinephrine (Sigma) added to the aggregometer cuvette 20 to 30 sec before introduction of the inducer. ATP release was measured using luciferin-luciferase (Chrono-lume reagent, Chrono-log) and an ATP standard from the same company. All parameters were recorded on an IBM AT computer using the Aggro-Link interface (Chrono-log).

The results were analyzed statistically by nonparametric (distribution-free) methods, i.e., methods that do not depend on the form of data distribution.

RESULTS

Immobilization resulted in a decreased relative weight of the thymus and in adrenal hypertrophy, but these changes reached highly significant levels (33%, p...0.001, and 34% $p<0.001$, respectively) only in rats immobilized for 24 h. The relative weight of the spleen also decreased, but only by 10%. This, plus the absence of lesions in the gastric mucosa, indicated that the stress reaction was relatively mild.

As shown in Table 1, the 24-h IS led to a reduced rate of platelet aggregation with any dose of ADP (except 1 μmol/liter) or collagen. In controls 2 (rats deprived of food and water for 24 h), the platelet aggregation parameters did not differ from their values in controls 1. The maximum amplitudes of platelet aggregation were also similar in these two groups (data not shown).

Epinephrine did not induce platelet aggregation, but at 1 mmol/liter increased significantly ($p<0.05$) both the amplitude and the rate of platelet aggregation induced by the two collagen doses (2 or 4 μg/ml) and by the lowest ADP dose (0.25 μmol/liter) in rats stressed by immobilization for 24 h. In rats so stressed for 6 h, only a tendency to increased platelet aggregation was noted after the addition of epinephrine (Table 1). These effects were not observed in the control groups.

The test and control groups did not differ in the amount of ATP released under the action of the inducers in the doses used (Table 2).

In the control groups, the behavior of rats in the open field virtually did not correlate (as shown by Kendall's test) with the parameters of platelet aggregation. In the group of rats stressed by 24-h immobilization, the relative weight of the thymus showed a positive correlation with the platelet aggregation rate in the presence of 4 μg/ml collagen ($p<0.016$) and with open-field behavioral parameters such as the number of squares crossed ($p<0.002$), the number of upright postures ($p<0.004$), and the number of times the rats looked into the "burrows" ($p<0.004$). These behavioral parameters also correlated significantly with the platelet aggregation rate in the presence of 2 μg/ml collagen. In ad-

TABLE 2. Release of ATP (nmol/liter per 10^{11} cells) from Dense Platelet Granules. The Values are Means±SEM

Preliminary addition of epinephrine (mmol/liter)	Inducer	Controls 1	Controls 2 (food- and water-deprived rats)	Rats stressed by immobilization	
				for 6 h	for 24 h
		ADP (μmol/liter)			
None	5.0	192.0±37.2 (14)	174.4±61.6 (7)	193.2±116.9 (5)	196.1±52.0 (11)
1.0	5.0	–	–	–	113.1±3.2 (2)
0.1	5.0	185.9±37.2 (4)	–	294.8±141.1 (3)	–
		Collagen (μg/ml)			
None	2.0	119.0±25.3 (15)	94.4±45.8 (6)	181.1±54.0 (8)	88.9±14.1 (11)
1.0	2.0	131.2±21.7 (7)	94.1±15.9 (2)	–	169.6±42.3 (3)
0.1	2.0	105.6±41.0 (5)	–	105.9±22.9 (5)	74.5±15.9 (2)
None	4.0	159.1±29.4 (14)	103.8±17.8 (5)	168.5±46.9 (7)	128.2±37.7 (11)
1.0	4.0	111.3±14.4 (6)	169.8±66.0 (2)	–	154.9±43.4 (5)
0.1	4.0	229.6±69.6 (2)	–	98.2±42.1 (2)	–

dition, the number of squares crossed correlated with the rats of platelet aggregation induced by 5 μmol/liter ADP ($p<0.04$).

Thus, both the amplitude and the rate of platelet aggregation were reduced in rats stressed by 24-h immobilization. This effect was not associated with the prolonged (24 h) food and water deprivation, but did correlate with the involution of the thymus.

This study has revealed a significant association of the immobilization-induced changes in platelet responses with the initial behavioral characteristics of the stressed rats. Since platelet responses to the inducers after the 6-h immobilization did no differ from those in the control groups, and since enhanced platelet activation tends to occur after acute exposure to stressors [11], the changes described above appear to have occurred in phases. The results of our experiments suggest that the discrepancies among the reported data on alterations in platelet aggregation in response to stress [5,6,11,12] are largely due to differences in the intensity of exposure to the stressors used and in phases of the platelet reaction recorded, and also to the fact that different animal models were employed. The mechanisms by which platelet functions are altered in emotional stress should therefore be studied using reproducible animal models that permit monitoring of individual responses to stress.

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